

Evaluation of a luminescent ruthenium complex immobilized inside Nafion as optical pH sensor

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The development of an optical pH sensor based on immobilization of a luminescent ruthenium complex, $[\text{Ru}(\text{bpy})_2(\text{dhphen})]^{2+}$ (bpy = 2,2'-bipyridine, dhphen = 4,7-dihydroxy-1,10-phenanthroline) in Nafion film is described. The sensor has a useful pH range of 1–8. The typical response time of the sensing film is 2–5 min. The polyanionic nature of the Nafion film eliminates interference from anions. The sensor also shows minimal interference effects from alkali metal and divalent cations and has good photostability and reproducibility.

There has been much interest in the development of optical pH sensors (pH optodes) for biomedical applications.^{1,2} The advantage of optodes over conventional electrochemical sensors is obvious: no electrical connection to the patients is required which makes the measurements safer for human use. In addition, the use of plastic optical fibre allows a high degree of mechanical flexibility combined with a very small size and low cost. Optical pH sensors are based on pH dependent changes of the absorbance or luminescence of certain indicator molecules.³ For absorbance-based sensors, the color of the dye changes with pH. For luminescence pH sensors, the difference in luminescence properties of the conjugate acid–base pair of the dye is measured. In this analytical application, the luminescence method has the advantages of higher sensitivity and selectivity over the absorbance method. The major limitation of most pH optodes is the narrow pH range covered: most dyes are only sensitive to changes in pH values over 2–4 pH units. Recently, there has been considerable interest in extending the use of pH optodes in industrial and hazardous environments⁴ and the development of pH sensing dyes with wider working range of pH values is necessary.

A number of fluorescent dyes have been used as indicators for pH sensing. Fluorescein and its related compounds are widely used as pH probes and sensors.^{5–8} However, fluoresceins have small Stokes' shifts, overlapping pK_a values and limited photostability. Hydroxypyrenetrisulfonic acid is another class of dye commonly used for pH sensing. Its major advantages include high fluorescence quantum yield, visible excitation and emission wavelengths, and a large Stokes' shift. It has a pK_a of 7.3 which is ideal for physiological measurements.^{9,10} However, hydroxypyrenetrisulfonic acid is rather sensitive to changes in the ionic strength due to its high charge. A near infrared fluorescence probe for pH determination has also been reported.¹¹ The sensing dye is a carboxylated cyanine incorporated into Nafion films. Instead of using fluorescent dyes that are pH sensitive, a pH optode based on co-immobilization of a pH-insensitive fluorophore, eosin, and a pH-sensitive absorber, Phenol Red, on the optical fibre has been developed.¹² The fluorescence emission spectrum of eosin overlaps significantly with the absorption spectrum of the basic form of Phenol Red. Thus, as pH increases, the amount of energy transfer from the fluorescent dye to the pH-sensitive indicator will increase resulting in a diminution of the fluorescence intensity. In addition to organic dyes, a number of dyes based on luminescent transition metal complexes are potentially useful for pH

optodes. An electropolymerized cobalt porphyrin has been reported to be optically sensitive to pH changes over the pH range 8 to 12.¹³ Several luminescent pH sensors based on photoinduced electron transfer have been described by Grigg and co-workers. The luminescent complexes include tin(IV) aminoethyltetraphenylporphyrin derivatives,¹⁴ di(2,2'-bipyridyl)(5,5'-diaminomethyl-2,2'-bipyridyl)ruthenium(II)¹⁵ and *p*-tert-butylcalix[4]arene-linked ruthenium(II) tris(bipyridyl) complexes.¹⁶

We are interested in the development of optodes based on luminescent transition metal complexes.^{17–19} These dyes offer distinct advantages including absorption in the visible region, long excited state lifetimes, high quantum yield and photostability. We report here our investigation on a luminescent ruthenium bipyridyl complex with the 4,7'-dihydroxy-1,10-phenanthroline ligand immobilized into a Nafion film as an optical pH sensor. This ruthenium complex is strongly emissive, and the protons of the hydroxy groups on the phenanthroline ligand are acidic and hence the absorption and emission spectra of the complex are dependent on the pH of the environment. It is cationic and can be easily immobilized inside the polyanionic polymer film through electrostatic binding, making it a potentially useful dye for optical pH determination. In this study, we employed Nafion as the polymer support for the pH sensing dye. Nafion is a perfluorinated polymer consisting of a tetrafluoroethylene backbone with pendent side chains of perfluorinated vinyl ethers which terminate in the sulfonic acid group. The structure of Nafion is believed to consist of three regions: a hydrophobic fluorocarbon backbone; a hydrophilic sulfonate ionic clusters site; and an interfacial region between the hydrophobic region and the hydrophilic region.²⁰ This inhomogeneous structure of Nafion together with its well known stability and insolubility in aqueous medium makes it a suitable polymer support for pH sensors. The hydrophilic ionic clusters region allows easy access of the analyte to the sensor molecules, whereas the relatively hydrophobic interfacial region provides a suitable environment for accommodation of the hydrophobic bipyridyl ligands of the metal complex. A schematic diagram showing the incorporation of the ruthenium complex in Nafion is given in Fig. 1. During the preparation of this manuscript, an article was published on the development of pH sensor based on immobilization of $[\text{Ru}(\text{phen})_2(\text{dhphen})]^{2+}$ (phen = 1,10-phenanthroline; dhphen = 4,7-dihydroxy-1,10-phenanthroline) inside poly(ethylene oxide) linked cyclic methyl siloxane polymer.²¹

Experimental

Materials

Ruthenium(III) chloride (RuCl_3) was obtained from Strem (Newburyport, MA, USA). 2,2'-Bipyridine (bpy), 4,7-dihydroxy-1,10-phenanthroline and Nafion (5 wt% solution) were purchased from Aldrich (Milwaukee, WI, USA). *cis*-Dichlorobis(bipyridine)ruthenium ($\text{Ru}(\text{bpy})_2\text{Cl}_2$) was synthesized in our laboratory according to a method previously published.²² Sodium chloride and lithium chloride (analytical-reagent grade) were obtained from Mallinckrodt (St. Louis, MO, USA) and Merck (Elmsford, NY, USA), respectively. All other chemicals were of analytical-reagent grade and were used without further purification. Oxygen and nitrogen gases (99.9%) were purchased from Hong Kong Oxygen (Hong Kong). Buffer solutions, prepared according to a literature method,²³ were measured by a Corning (Corning, NY, USA) Model 7 pH meter prior to use.

Synthesis of the dye

$[\text{Ru}(\text{bpy})_2(\text{dhphen})](\text{ClO}_4)_2$ [caution: all perchlorate salts are potentially explosive!] was synthesized by procedures reported by Giordano *et al.*²⁴ with a slight modification: $\text{Ru}(\text{bpy})_2\text{Cl}_2$ (0.5 g) and dhphen (0.25 g) were refluxed in 1 : 1 (v/v) ethanol–1.0 M NaOH for 2 h. The dark red solution was then neutralized to pH 6 by HCl and any excess ligand was filtered off. The product was precipitated from an aqueous solution of pH 2 by addition of LiClO_4 . The crude product was purified by recrystallization from MeOH to give reddish brown crystals. The purity of the complex has been checked by elemental analysis and the C, H, N ratio of the compound synthesized is consistent with the empirical formula $\text{C}_{32}\text{H}_{24}\text{Cl}_2\text{N}_6\text{O}_{10}\text{Ru}$.

Preparation of pH sensing film

A 0.3 mM solution of the $[\text{Ru}(\text{bpy})_2(\text{dhphen})](\text{ClO}_4)_2$ complex was prepared in analytical-reagent grade ethanol. Nafion obtained as 5 wt.% solution was diluted five fold with ethanol. A complex–polymer mixture was prepared by mixing an appropriate amount of the above two solutions thoroughly. A 0.4 ml aliquot of the resulting mixture was placed on the surface of a glass slide (0.9 cm $\times$ 5 cm), spin-coated and air-dried. Coatings prepared in this way have an estimated thickness of

about 5 μm .²⁵ The coated glass slide was then inserted into a 1 cm pathlength polystyrene cuvette containing buffer solution for spectroscopic measurements.

Instrumentation

UV/VIS absorption spectra were recorded on a Milton Roy (New York, NY, USA) 3000 photodiode array spectrophotometer. Luminescence measurements were made either on a Spex (Edison, NJ, USA) 'Fluorolog-2' Spectrofluorometer or on a Perkin-Elmer (Norwalk, CT, USA) LS-50B spectrofluorometer. All luminescence measurements were performed in an aerated buffer solution. The response time of the sensing film was measured by pumping different buffer solutions into and out of the cuvette with a Cole-Parmer (Vernon Hills, IL, USA) Model 7550 peristaltic pump. Unless otherwise stated, all measurements were made at room temperature ($25 \pm 2^\circ\text{C}$) and atmospheric pressure.

Results and discussion

Optical characteristics of the pH sensing film

The absorption spectra of $[\text{Ru}(\text{bpy})_2(\text{dhphen})]^{2+}$ immobilized in Nafion in different acid and base media are shown in Fig. 2. The spectral change is a result of an acid–base equilibrium of the complex as presented in Scheme 1.²⁴ The spectral changes are completely reversible with variations in the pH. The absorption spectra show two isosbestic points at around 415 and 475 nm, respectively. As the absorption spectra overlap more precisely at 415 nm, this wavelength was chosen as the excitation

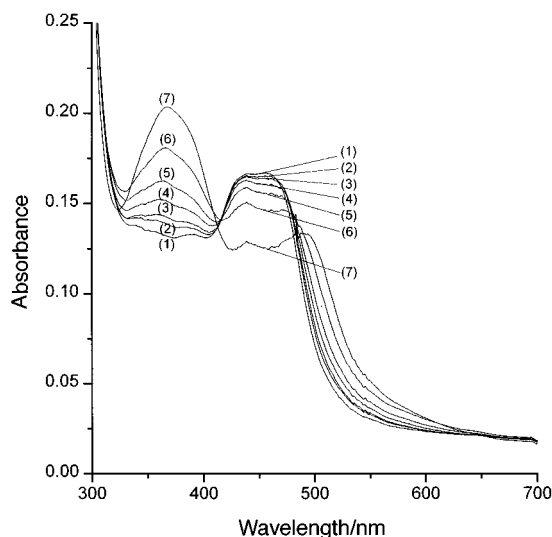


Fig. 2 Absorption spectra of $[\text{Ru}(\text{bpy})_2(\text{dhphen})]^{2+}$ entrapped within Nafion film in different pH buffers. Curves 1–7 are at pH 1.12, 4.25, 5.01, 6.02, 6.89, 7.95 and 8.83, respectively.

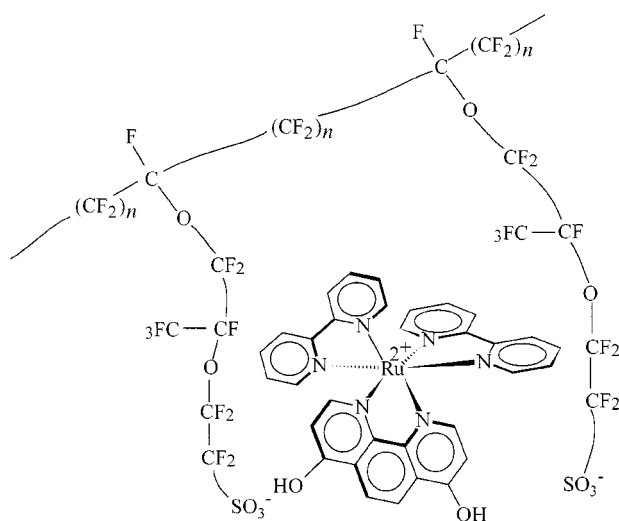
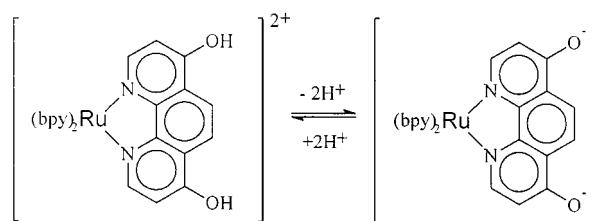


Fig. 1 A schematic diagram showing a ruthenium complex incorporated inside Nafion.



Scheme 1

wavelength in subsequent luminescence measurements which will result in more accurate pH measurements. Both the protonated and the deprotonated forms of the complex can emit light in aerated aqueous solution, though the latter is much less emissive. Since the intensity of emission depends on the amount of light absorbed by the emitting species, the excitation has to be made at a wavelength (the isosbestic point) at which both the protonated and the deprotonated complexes absorb the same amount of light. Under such conditions, the emission intensity measured will not be affected by the difference in absorbance of the two emitting species. Excitation of the complex results in the emission spectra shown in Fig. 3(A). The emission intensity of the complex decreases with increase in pH. Plots of the emission intensity *versus* pH for the complex in solution and after immobilization into Nafion are shown in Fig. 3(B). The emission intensity decreases gradually as the pH of the solution increases and levels off beyond pH 8. Although the complex possesses two acidic protons, previous studies indicated that the first and second acid dissociation constants, pK_{a1} and pK_{a2} , of this complex are nearly the same,²⁴ and hence only one inflection point is observed in the plots of change in emission intensity *versus* pH. In solution, the inflection point occurs at about pH 2.6. After incorporation inside Nafion, the inflection point was found at around pH 5.1. It is interesting to note that after immobilizing the ruthenium complex into Nafion, the apparent pK_a is higher than in solution. Increase in apparent pK_a

after incorporation into Nafion films has also been observed in other metal complexes.²⁶ It could be due to the polyanionic nature of the Nafion matrix which tends to stabilise the more highly charged, acidic form of the acid–base pair of the dye. The sulfonic sites (SO_3^-) of Nafion can attract protons and hinder the entrance of anionic buffers such as phosphate and acetate that make the environment inside the film more acidic than the outside buffer solution. As a result, the apparent pK_a value of the complex is increased after immobilization in Nafion.

Response time and reproducibility

The response time of the sensing film was measured by replacing the buffer solutions in the cuvette rapidly with the use of a peristaltic pump. The flow rate of the peristaltic pump was set at 40 ml min^{-1} so that it takes only about 10 s to replace all the solution in the cuvette. The time taken to replace the buffer is very short compared to the response of the sensor, so the interruption did not affect the measurement of the response time significantly. The change in emission intensity at 612 nm was then monitored under static conditions. A typical response curve of the sensor to the change in pH is shown in Fig. 4. The signal changes are fully reversible. The response time is taken to be the time required for 95% of the total signal change. The response time depends on the magnitude of the change in pH and is about 320 s when going from pH 2.47 to 5.37. When going from pH 3.38 to 1.12, the response time was 165 s. The signal levelled off after equilibrium had been reached and no drift in response was observed under the experimental conditions employed.

Reproducibility of the sensor was evaluated by measuring the emission intensity consecutively in pH 2.47–6.02 buffer solutions. The reproducibility for pH measurement is reasonably good with standard deviation in the range of 0.04 to 0.11 pH units. A summary of the reproducibility of the sensor under different pH conditions is given in Table 1.

Sensitivity and interferents

The sensitivity limit of the sensing film, taken as three times the noise level, is about 0.1 pH unit. This is comparable to the conventional pH electrode. As Nafion is a polyanionic polymer, anions are excluded from the sensing film due to electrostatic repulsion, whereas cations can enter the film and affect the response of the sensor. The responses of the sensing film in the presence of alkali metal and divalent transition metal ions are

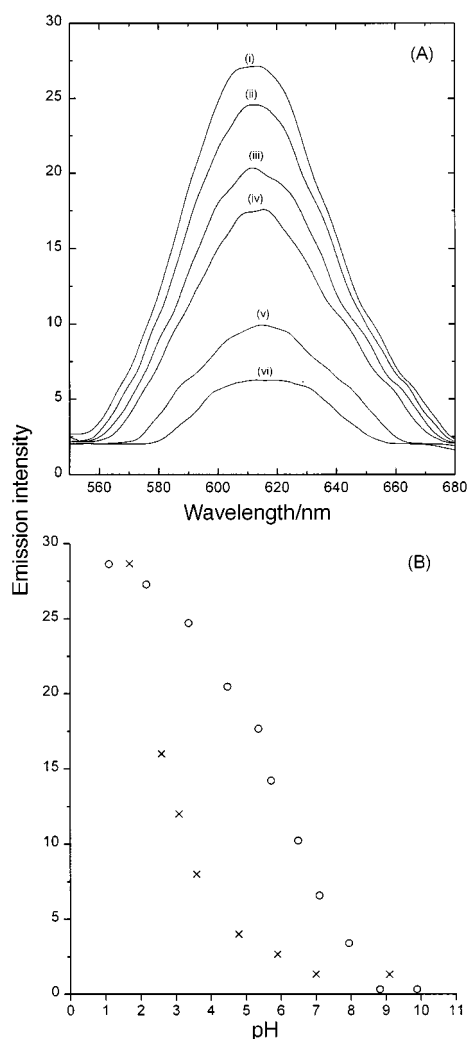


Fig. 3 (A) Emission spectra of $[Ru(bpy)_2(dhphen)]^{2+}$ entrapped within Nafion film in different pH buffers with excitation wavelength set at 415 nm. Curves 1–6 are at pH 2.18, 3.38, 4.49, 5.37, 6.50 and 7.11, respectively. (B) Plots of emission intensity *versus* pH for the ruthenium complex in solution ($\times$) and after immobilisation into Nafion ($\circ$).

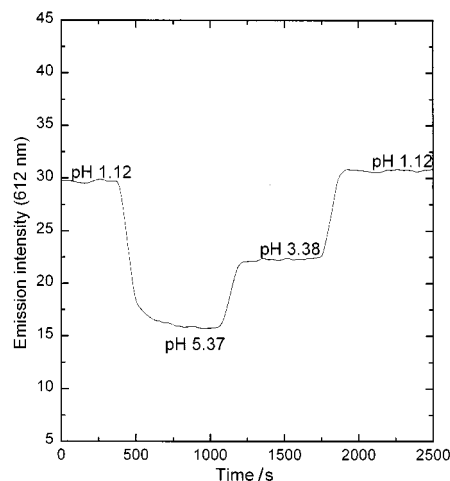


Fig. 4 Response of the sensing film towards changes in pH. The excitation wavelength is 415 nm.

shown in Fig. 5(A). At low concentration, the monovalent and divalent metal ions have little effect on the luminescence intensity of the sensor. When the concentration of the metal ions reaches about 1 M, a small decrease in the luminescence intensity (less than 8% change) is observed. The interference effect of the cations is also reflected in the change in ionic strength of the buffer solutions. As shown in Fig. 5(B), there is a reduction in the emission intensity as the ionic strength of the buffers increases.

The effect of oxygen on the response of the sensor was also studied as oxygen is a well known quencher for luminophores. Fig. 6 shows the response of the sensing film when changing a

buffer solution saturated with oxygen to a completely deaerated solution. The emission intensity decreases by 14% from a pure nitrogen to a pure oxygen environment. The extent of oxygen quenching was found to be more or less the same in all buffer solutions. Although oxygen is an interferent, this would not cause significant error for measurements on aerated solutions in which the oxygen concentration is constant. However, it was found that the sensor is more sensitive towards pH changes under deaerated conditions. In a nitrogen saturated environment, the average change in the emission intensity is about 4.6 units per unit change in pH. When the measurement is taken under aerated conditions, the change in emission intensity unit per unit pH drops to about 4.1, representing an 11% reduction in sensitivity.

Table 1 Reproducibility of the sensor in different pH buffers

pH	Emission intensity ^a	± intensity (s) ^b	± pH (s)
2.47	27.24	0.27	0.11
3.38	24.29	0.22	0.06
4.49	19.51	0.21	0.04
5.37	15.53	0.25	0.05
6.02	10.91	0.25	0.04

^a Mean (*n* = 5). ^b s = standard deviation.

Sensor stability

The sensing film was usually kept in de-ionized water before use to prevent it from drying out. One problem with immobilised dyes is the leaching of the dyes into the solution. Leaching of the dye from the Nafion film was monitored by soaking the film in buffer solution continuously for two weeks and recording the emission spectrum daily. The dye was found to be stable inside the Nafion film over pH 1–9 and very little leaching of the dye was observed. The emission spectrum after two weeks was almost the same as the original spectrum recorded on the first day with little change in the emission intensity. However, substantial leaching of dye was observed in basic medium with pH > 10. This is due to the loss of electrostatic interaction between the dye and the Nafion matrix as the immobilized complex is fully deprotonated and becomes neutral in basic media. As a result, the use of this sensor for pH measurement is limited to pH < 9.

The photostability of the sensor was also examined by illuminating the film continuously in the Perkin-Elmer LS-50B spectrofluorometer with a 20 kW (8 μs duration) xenon discharge lamp for 2 h with the excitation bandwidth set to 15 nm. No diminution in emission intensity was observed after the experiment indicating that the photostability of the sensor is reasonably good.

The luminescence intensity of the sensor decreases with increasing the temperature of the sample solution. The luminescence intensity of the sensor decreases by about 11% when the temperature of a buffer solution of pH 3.38 is raised from 21 to 34 °C. This is consistent with an increase in radiationless decay with the temperature.

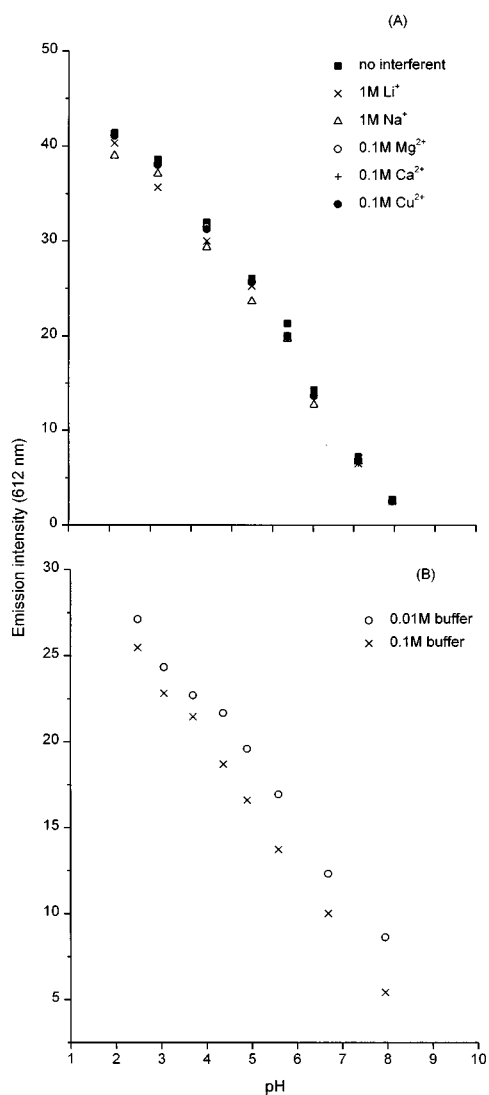


Fig. 5 Plots of emission intensity versus pH for the sensing film (A) in the presence of different metal cations and (B) in buffers of different ionic strength.

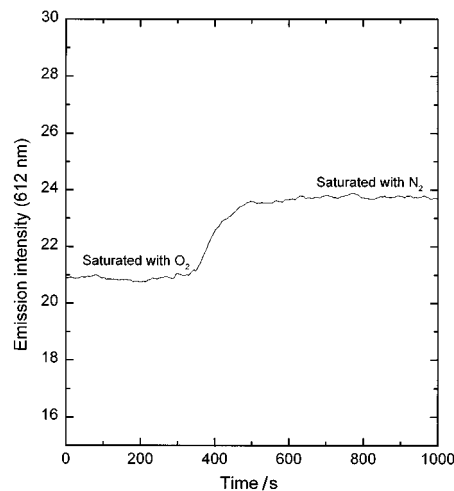


Fig. 6 Response of the sensing film to oxygen. The solution is a pH 4.49 buffer solution. Excitation wavelength: 415 nm.

Conclusions

We have demonstrated that the luminescent $[\text{Ru}(\text{bpy})_2(\text{dphen})]^{2+}$ complex immobilized in Nafion film can be used as a luminescence-based pH sensor. The sensor exhibits a large dynamic range of 8 pH units (pH 1–8). This sensor exhibits advantages over many existing optical pH sensors including wider dynamic pH range, ease of fabrication, good photostability and minimal interference from metal cations. The application of this sensor for pH monitoring of industrial processes is in progress in our laboratory.

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